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Addition of a Magnetite Layer onto a Polysulfone Water Treatment Membrane to Enhance Virus Removal

by

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ABSTRACT

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The applicability of low-pressure membranes systems in distributed (point of use) water treatment is hindered by, among other things, their inability to remove potentially harmful viruses and ions via size exclusion. According to the USEPA and the Safe Drinking Water Act, drinking water treatment processes must be designed for 4-log virus removal. Batch experiments using magnetite nanoparticle (nano-Fe_3O_4) suspensions and water filtration experiments with Polysulfone (PSf) membranes coated with nano-Fe_3O_4 were conducted to assess the removal of a model virus (bacteriophage MS2). The membranes were coated via a simple filtration protocol. Unmodified membranes were a poor adsorbent for MS2 bacteriophage with less than 0.5-log removal, whereas membranes coated with magnetite nanoparticles exhibited a removal efficiency exceeding 99.99% (4-log). Thus, a cartridge of PSf membranes coated with nano-Fe_3O_4 particles could be used to remove viruses from water. Such membranes showed negligible iron leaching into the filtrate, thus obviating concern about colored water. Further research is needed to reduce the loss of water flux caused by coating.

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LIST OF SYMBOLS AND ABBREVIATIONS

$AlCl_3$	Aluminum chloride
BET	Brunauer, Emmet and Teller method
$CaCl_2$	Calcium chloride
°C	Celsius degree
CFU	Colony Forming Units
DBPs	Disinfection-byproducts
DI	Deionized
g	Gram
GFD	Gallons per square foot per day
h	Hour
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPM	High pressure membrane
IEP	Isoelectric Point

L	Liter
LMH	Liters per square meter per hour
LPM	Low pressure membrane
m^2	Square meter
m^3	Cubic meter
M	Molar concentration
MF	Microfiltration
mg	Miligram
min	Minute
mL	Mililiter
mm	Milimeter
mM	Milimolar concentration
mV	Milivolt
µg	Microgram
µL	Microliter
µm	Micrometer

NaCl	Sodium Chloride
nano-Fe_3O_4	Magnetite nanoparticles
NaOH	Sodium hydroxide
NF	Nanofiltration
n-Fe_3O_4-PSf	Nanomagnetite-coated polysulfone
nm	Nanometer
NOM	Natural Organic Matter
PFU	Plaque Forming Units
ppm	parts per million
PSf	Polysulfone
psi	pounds per square inch
rpm	revolutions per minute
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
s	second
TMP	Transmembrane pressure

UF	Ultrafiltration
USEPA	United States Environmental Protection Agency
UV	Ultraviolet light
WHO	World Health Organization
USEPA	United States Environmental Protection Agency

CHAPTER 1:

INTRODUCTION

According to the World Health Organization, one-sixth (1.1 billion people) of the world's population lacks access to adequate water supplies (WHO, 2007). An estimated 31 million people contract virus-related gastrointestinal illnesses each year, of which only about 9 million are food-related (Mead et al., 1999). Groundwater, previously thought to be naturally pristine, has been found to contain enteric viruses (transported from faulty septic tanks, landfills, fields treated with waste sludge, latrines, or contaminated waterways), even in confined aquifers (Borchardt et al., 2007). Enteric viruses have also been detected in treated drinking water (Ehlers et al., 2005; Keswick et al., 1984; Lee and Kim, 2002; Vivier et al., 2004) and recent studies indicated that enteric viruses were the leading causative agents of waterborne diseases in the USA and worldwide (Fong and Lipp, 2005; Griffin et al., 2003). Therefore, the presence of enteric viruses in drinking water sources is a growing public health concern necessitating an effective, simple removal technology.

1.1 Membrane Technology for Water Treatment

Membrane treatment processes are considered a promising innovative technology to meet the current and future water treatment regulations. The goal is to ensure proper removal of pathogens, ion-sized particles and organic matter, desalination, and wastewater treatment for reuse.

The main advantages of membrane processes are the production of high quality water without chemicals or utilities, and small footprint (Pronk et al., 2009).

As an alternative to conventional water treatment the application of membrane technology in water has increased dramatically in the last decade (Furukawa, 2008). New developments in membrane technologies (e.g., materials) resulted in the decreasing of membrane costs and energy requirements (Churchhouse, 2000).

Membrane filtration technologies employed in water treatment are classified in microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The main mechanism to remove water contaminants is size exclusion. Based on the operating transmembrane pressure (TMP), membranes employed in water treatment can be classified as Low-pressure membranes (LPM) and High-pressure membranes (HPM). Typically LPMs operate at TMPs less than 1 to 2 bar. LPMs include microfiltration and ultrafiltration.

The use of low-pressure membranes is growing worldwide. Their application in drinking water treatment and wastewater reuse accounted for 82% of the total installed capacity of LPM systems (Figure 1) (Furukawa, 2008). Low-pressure membranes processes (MF and UF) are capable of removing suspended or colloidal particles. Generally these membrane media are manufactured in hollow-fiber modules. The pore sizes of MF membranes range from 0.1 – 0.2 μm. For UF, pore size generally range from 0.01 – 0.05 μm or less. The small pore size ensures high log-removal of microbial pathogens such as protozoan parasites (e.g., *Cryptosporidium* and *Giardia*) and bacteria (Hagen, 1998). Significant virus removal can be achieved using UF since their size ranges from 20-200 nm. Some recent limitations with respect to bacterial retention are reported for MF membranes (Wang et al., 2007). Overall the application of these technologies faces some limitations that include their inability to remove ionic species and nano-sized particles such as viruses.

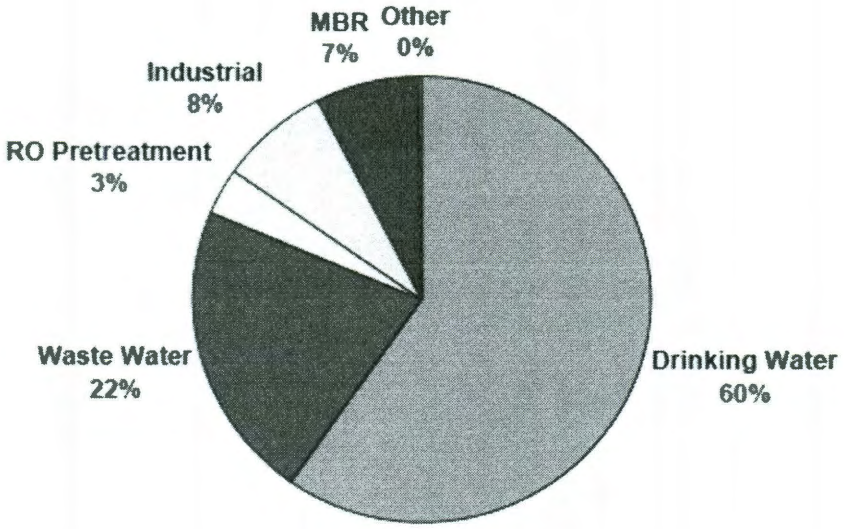

Figure 1. Global Installations for Low Pressure Membranes by Application (Furukawa, 2008).

1.2 Background on Viruses

Viral particles are small (20-200 nm in size) and enteric viruses (e.g., *Norovirus,* hepatitis A virus*, Enterovirus*), which are usually sized 20-30 nm diameter, are among the most difficult water-borne microorganisms to remove (Langlet et al., 2009). The virion of most enteric viruses consists of nucleic acid genome encapsulated by a capsid composed of proteins containing weak acid and base groups (e.g., carboxyl, sulfhydril and amine groups) that are ionizable (Brown and Sobsey, 2009). In natural aquatic environments viruses are charged biocolloidal particles with the ability to adsorb to solid surfaces, which influences viral fate and transport. The factors controlling the adhesion kinetics of viruses including the type of viruses and the associated surface properties are pH, ionic strength, degree of water saturation in soil, and the presence or absence of interfering substances such as natural organic matter (NOM), which may either adsorb the viruses or compete with them for adsorption sites on a surface (Bitton et al., 1976;

Chu et al., 2003; Hurst et al., 1980; John and Rose, 2005; Schijven and Hassanizadeh, 2000).

The net charge of a virus depends on the pH of the medium and the surface chemistry of the virus. The isoelectric point (IEP) is specific to the individual virus type and strain. Typically viruses have IEP in the range of 3-7 (Dowd et al., 1998); thus, they may be either positively or negatively charged in natural waters (pH 4-9). In most cases, viruses are negatively charged and positively charged surfaces can adsorb and possibly inactivate them in aqueous systems based on electrostatic interactions.

1.3 Nanotechnology and Water Treatment

Conventional water treatments methods have some limitations such as (Brame and Alvarez, 2011):

- Large scale infrastructure and material required.
- Generation of harmful disinfection-byproducts (DBPs).
- Pathogens resistance to disinfectants.
- Over time the water quality standards are becoming stricter and new contaminants are emerging.
- Use of aging water distribution infrastructure.

Nanotechnology has been identified as a promissory alternative to face some challenges to provide safe drinking water. The potential impact areas for nanotechnology in water treatment include three categories: (i) Treatment and Remediation, (ii) Sensing and detection and (iii) Pollution prevention (Theron et al., 2008).

Due to their large superficial area and high reactivity, many natural and engineered nanomaterials have excellent properties as adsorbents, catalysts, antimicrobials and sensors. Some antimicrobial nanomaterials include chitosan, silver nanoparticles (nAg), photocatalytic TiO_2, fullerol, aqueous fullerene nanoparticles (nC60) and carbon nanotubes (CNT) (Li et al., 2008). The incorporation of this nanomaterials in conventional or new technologies for water treatment is an innovative approach that enhances disinfection avoiding the production of disinfection byproducts and ineffectiveness to disinfectant-resistant pathogens.

1.3.1 Metal and Metal Oxides Nanoparticles

Silver nanoparticles (nAg)

Although silver compounds have been well known for long time to exhibit high toxicity toward microorganisms (Liau et al., 1997), it was only recently that silver nanoparticles started to be studied as antimicrobial agents towards bacteria (Sondi and Sondi, 2004, Singh et al., 2008) and viruses (Elechiguerra et al., 2005, Morones et al., 2005). The results indicate a stronger biocide effect on Gram-positive than against Gram-negative bacteria. The bactericidal efficacy of Ag nanoparticles seems to be dependent on particle size and shape. Regarding size, small particles (<10 nm) were found to be most effective toward certain bacteria among particles with size between 1-100 nm (Morones et al., 2005). Similarly, nanoparticles ranging from 1 to 10 nm inhibit certain viruses from binding to their host cells (Elechiguerra et al., 2005). Concerning shape, truncated triangular plates displayed the best biocide activity when compared to spherical or rod-shaped nanoparticles, probably due to differences in their active surface area (Pal et al., 2007). The exact mechanism of the bactericide effect of nAg is still in debate, but some of the mechanisms proposed are the penetration of the bacterial cell wall, membrane damage through

formation of free radicals, and damaging interactions of silver nanoparticles with either sulphur-containing proteins or DNA (Theron et al., 2008).

Titanium Dioxide (TiO_2)

During the last two decades, abundant information has been collected regarding the photocatalytic inactivation of bacteria by TiO_2 (Wei et al., 1994; Watts et al., 1995; Kikuchi et al., 1997; Cho et al., 2005; Benabbou et al., 2007; Page et al., 2007). It has also been found that nano-sized TiO_2 can inactivate viruses such as MS2 bacteriophage (Cho et al., 2005) among others. Its mode of action involves generation of reactive oxygen species (ROS) under UVA light (Kikuchi et al., 1997), which causes oxidative stress in the microorganisms and leads to cell death. In order to improve light absorbance of TiO_2, doping with noble metals is currently explored. In particular, it was recently demonstrated that doping TiO_2 with silver caused a remarkable improvement in viral inactivation (Liga et al., 2011). A recent approach is to couple the photocatalytic microbial effect of TiO_2 with membrane separation technology. This photocatalysis-membrane coupling technology has shown not only to preserve the functions of the two separate processes but also some synergistic effects, although many challenges still need to be solved (Xiao et al., 2010).

Zinc Oxide (ZnO)

Like TiO_2, zinc oxide also exhibits strong UV light absorption. Likewise, it has shown high bactericide activity against a broad spectrum of microorganisms (Huang et al., 2008). However, the mechanism of its antimicrobial activity and the effect of nanoparticle size is still under study (Raghupathi et al., 2011, Li et al., 2011). The suggested bactericidal mechanisms include

generation of ROS, deposition of the nanoparticles on the surface of bacteria, accumulation of the nanoparticles in the cytoplasm or in the periplasmic region, and generation of Zn^{2+} ions or Zn^{2+} labile complexes. Zinc oxide nanoparticles have also been used to remove arsenic from water, even though bulk zinc oxide cannot absorb arsenic (Tiwari et al., 2008).

Iron Oxides

Several nano-sized iron containing minerals, including hematite (Fe_2O_3) and magnetite (Fe_3O_4), have been studied for adsorption processes in wastewater treatment. The small size of those nanoparticles, typically 2-3 orders of magnitude smaller than bacteria, provides a much larger surface area than ferric oxide typically used in water treatment. The magnetic iron oxide minerals are collectively known as ferrites. Various ferrites, including magnetite, were evaluated in batch modes for actinide and heavy metal removal in wastewater. Also, ferrites have been employed to enhance removal of cobalt and iron from simulated groundwater (Tiwari et al., 2008). More recently, iron oxides have found application in virus removal for drinking water, but that will be discussed in more detail in the following section.

1.3.2 Antiviral Properties of Iron Oxides Particles

Virus adsorption onto different solid surfaces such as hematite, clays, activated carbon, ceramic modified materials and iron oxides/hydroxide species is well documented. Nonpathogenic model viruses, such as bacteriophages MS2, PRD1 and phi X174, and the enteric virus *Rotavirus* have been used to investigate virus transport in different media. Viruses are known to adsorb onto iron oxides commonly present in soil and artificially incorporated into filtration media. Magnetite sand and hematite particles have been shown to be effective filtration media for poliovirus

removal (Moore et al., 1981). Ryan et al. (2002) also reported increased removal of PRD1 and MS2 in a sand column by coating quartz sand with iron oxides. Effluent analysis indicated that the viruses had attached strongly to the medium, the viruses had been inactivated (potentially by the strong attractive force between the capsid and ferric oxyhydroxides), and the remaining virus nucleic acids were released into the effluent. Chu et al. (2003) found that viruses are removed effectively in soils that contained iron oxides, and that the most influential environmental factors for virus removal in addition to the presence of iron were pH, NOM, metal oxides contents and soil saturation with water. Bitton et al. (1976) suggested that magnetite was a good adsorbent even at low concentrations (300 ppm) and considered various environmental factors affecting adsorption of poliovirus in water and wastewater onto magnetite. Rao et al. (1981) used magnetite in conjunction with pH adjustment (to pH 3) and 0.0005 M $AlCl_3$ to effectively adsorb and concentrate poliovirus I for coagulation. High retention capacities for MS2 have been reported using magnetite treated with successive cycles of acid and alkali washing (Atherton and Bell, 1983).

NOM in particulate and dissolved form decreases the retention capacity of soil for MS2. Gutierrez et al. (2009) showed high removal of Rotavirus and MS2 by glass fiber coated with hematite nanoparticles in batch and flow-through experiments. However, virus adsorption decreased in the presence of NOM and bicarbonate ions. Modified media such as ceramics containing Fe and Al oxides can enhance the virus adsorption and inactivation through sorption processes (Brown and Sobsey, 2009).

Overall, these past studies show that incorporating iron oxides into water filtration systems could

enhance virus removal. Furthermore, manipulations to develop positive surface charges from the protonation of iron oxides (e.g., by decreasing pH) can facilitate electrostatic attraction of negatively charged viruses and enhance their removal efficiency. However, the potential of virus removal by incorporating iron oxides into polymeric low-pressure membranes has received limited attention, and little is known about the effect of water chemistry on this approach.

This study considers the incorporation of magnetite nanoparticles (nano-Fe_3O_4) into polymeric microfiltration membranes, creating a one-step treatment for virus removal. Two types of experiments were conducted at bench scale (1) Batch adsorption experiments using magnetite nanoparticles suspensions to assess virus adsorption capacity in the presence of common inorganic ions (Na^+ and Ca^{2+}) on virus adsorption capacity, and (2) Membrane filtration experiments using polysulfone membranes coated with nano-Fe_3O_4 to assess virus removal efficiency and iron leaching. Results suggest that nano-Fe_3O_4 coated membranes could potentially be used in point of use devices or small membranes systems for virus removal to avoid formation of harmful disinfection by products associated with the use of chemical disinfectants.

CHAPTER 2*:

MATERIALS AND METHODS

2.1 Membrane Coating with nano-Fe_3O_4

Nanomagnetite-coated polysulfone membranes (nFe_3O_4-PSf) were synthesized as follows. Samples of a commercial polysulfone membrane (0.2 μm mean pore size, 47 mm diameter, HT Tuffryn; Pall Co.) were cut to coupons of 25 mm in diameter and soaked in 100% ethanol solution for 10 minutes to fully wet the membrane. Then magnetite nanoparticles (Sigma-Aldrich, nanopowder <50 nm particle size, BET surface area > 60 m^2/g, ≥ 98% purity) were coated onto the membrane surface by filtering 3 mL of a nanomagnetite suspension in ethanol at a concentration of 1 g/L through the membrane at a flow rate of ~1 mL/min. This resulted in a total iron content of 3.9% by weight. Prior to filtration, the nanomagnetite suspension was sonicated for 5 minutes using a probe sonicator (Sonic Ruptor 250 Ultrasonic Homogenizer, Omni International; Kennesaw, GA) and for 10 minutes using a bath sonicator (Branson Ultrasonic 5510; Danbury, CT) to ensure homogeneous magnetite nanoparticle dispersion. The membranes without rinsing were then dried in the oven at 100 °C for 30 min and stored at 4 °C. To assess the capacity of the membrane to retain the nanomagnetite particles on the membrane surface and in membrane pores during typical microfiltration processes, the coated membrane samples were subject to two different rinsing protocols: superficial rinsing thoroughly with deionized (DI) water for 5 min and/or transversal rinsing by filtering 30 mL of DI water through the membrane for 10 min. These two rinsing protocols simulate the hydraulic condition encountered during cross-flow and dead-end filtration, respectively.

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* The information presented here concerning the addition of nanomagnetite into a polysulfone membranes has been accepted for publication in I. Raciny, K. R. Zodrow, D. Li, Q. Li, and P. J. J. Álvarez. Addition of a Magnetite Layer onto a Polysulfone Water Treatment Membrane to Enhance Virus Removal. *Water Science and Technology*. (In press).

2.2 Membrane Characterization

Permeability and Contact Angle. Membrane permeability was determined by measuring the deionized water flux at room temperature in an Amicon Stirred cell over a working pressure range of 5-25 psi. The flow was measured using a digital scale that monitors cumulative permeate volume as a function of time. Membrane hydrophobicity was assessed by sessile drop contact angle measurement of DI water using a contact angle analyzer (DROPImage Standard).

Iron Concentration in the Permeate. Iron leeching from the membrane was evaluated by analyzing the effluent (permeate) for total iron concentration using inductively-coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 4300DV, Norwalk, CT). Samples (4 mL) were preserved with 1% HNO_3 prior to analysis.

2.3 Virus Analysis

Preparation and Quantification of MS2. Bacteriophage MS2 (ATCC#15597-B1) was used as a model waterborne virus. MS2 has capsid properties similar to those of poliovirus (Badireddy et al., 2007) and is commonly used as a surrogate to evaluate human enteric virus removal (You et al., 2005). MS2 is a non-enveloped icosahedral single-strand RNA coliphage, with a diameter of 26.0-26.6 nm (VanDuin, 1988) and an isoelectric point of 2.2-3.9 (Gutierrez et al., 2009; Yuan et al., 2008; Zerda et al., 1985). A low isoelectric point indicates a high net negative charge on the virus surface at typical pH values of natural water. Langlet et al., (2009) discussed the physico-chemical characteristics of MS2 phage, identifying it as a worst-case scenario for the evaluation

of virus removal by membrane filtration (*i.e.,* (i) small size, (ii) high negative surface charge and (iii) high degree of hydrophobicity).

MS2 was propagated according to the method described by Zhu et al. (2005). To propagate MS2, the bacteriophage (100 μL) was incubated with 100 μL of its *Escherichia coli* host (ATCC 15597) with a concentration of $4x10^8$ CFU/mL for 10 min in 900 μL 0.1 M bicarbonate buffer (pH 8.3). Then, warm tryptic soy soft agar was added to the suspension and the mixture was deposited onto a Luria-Bertani agar plate using the agar-overlay technique (Kennedy et al., 1986). After incubation overnight at 37 °C, the viruses were removed from the plate with bicarbonate buffer. Approximately 5 mL of the viral suspension was added to the plate and left to incubate for 10 minutes. This suspension was then removed and centrifuged at 5,000 rpm for 1 min, and the supernatant was filtered through a 0.2 μm polyethersulfone filter. The resulting viral suspension was stored at 4 °C until use. The viral stock concentration was determined by the standard Plaque Forming Units (PFU) assay (ISO-10705-1).Viruses were detected by the formation of clear zones (plaques) on the bacterial mat. Dilutions exhibiting 20-300 plaques per plate were considered for MS2 enumeration. All virus assays were performed in duplicate, and the virus concentration was reported by averaging the number of plaques from two replicate plates. Removal was calculated as logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.

Virus Adsorption onto Magnetite Nanoparticles. Magnetite nanoparticles for batch adsorption experiments were purchased from READE advanced materials, Reno, NV. The nominal size range of the nanoparticles was from 20 nm to 30 nm. Surface area of the magnetite nanoparticles

was determined by BET surface analyzer to be 69.4 m^2/g. Suspensions of magnetite at different concentrations were prepared by adding different amounts of magnetite powder to 15 mL of 100 mg/L background electrolyte solution to a final concentration of 1 g/L. The pH of the suspension was then adjusted to pH 6 by adding 0.034 M of HCl or NaOH. The suspensions were ultrasonicated for 30 s using a bath sonicator (Branson Ultrasonic 5510; Danbury, CT) immediately prior to the adsorption experiments. To assess electrostatic attraction as a potential virus removal mechanism, the zeta potential of the magnetite nanoparticles in all test solutions was measured using a *ZetaSizer* Nano ZS (Malvern, Inc., Southborough, MA). The magnetite nanoparticles were found to be positively charged with the surface zeta potential in the range of +12.5 to +16 mV, suggesting electrostatic attraction between the magnetite nanoparticles and the negatively-charged MS2.

The MS2 stock was diluted into the magnetite suspensions to a final concentration of 10^6 PFU/mL in 20 mL glass vials. These vials were shaken at 250 rpm at room temperature for 1 h. At predetermined time intervals, 1 mL samples were taken from the suspension and placed into a 1.5 mL vial. A horseshoe magnet was placed under the vial to separate the magnetite nanoparticles from the aqueous phase. The supernatant was then serially diluted, and the virus titer quantified using the agar overlay technique. Control experiments were conducted simultaneously using four different buffer solutions at pH 6: 1.7 mM NaCl+1 mM $CaCl_2$, 1.7 mM NaCl+2 mM $CaCl_2$, 3 mM NaCl or 4 mM NaCl.

Virus Adsorption onto Magnetite-PSf Membranes. Membrane filtration experiments were conducted to evaluate the MS2 adsorption capacity of the polysulfone MF membranes coated

with magnetite nanoparticles (nFe_3O_4-PSf). For every test, 3 mL of viral suspension in 0.1 M bicarbonate buffer (adjusted to pH 8.3) were used. The viral suspension with a concentration between 10^6 and 10^7 PFU/mL was filtered at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter, corresponding to a volumetric flux of 3×10^{-3} m^3/m^2-min. Prior to each test the membrane sample was rinsed superficially and transversally by filtering DI water through the membrane. Samples of viral solution were taken from the feed (influent) and permeate (effluent) streams, and were subsequently serial-diluted according to the protocol described by Zodrow et al. (2009) and quantified by the PFU method. Each filtration experiment was carried out at least in duplicates. Control experiments for MS2 filtration on non-coated polysulfone membranes were performed to provide a baseline for virus removal by the PSf membrane alone.

A continuous flow experiment was also conducted to evaluate virus breakthrough at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter (volumetric flux of 3×10^{-3} m^3/m^2-min). About 20 mL of viral suspension was filtered continuously through the membrane, and 3 mL permeate samples were collected. Virus removal was calculated as logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.

CHAPTER 3:

RESULTS AND DISCUSSION

3.1 Virus Adsorption onto Magnetite Nanoparticles (Batch) Experiments

The effect of the divalent Ca^{2+} versus monovalent Na^{+} cations on MS2 removal was compared (at pH 6) to investigate the effect of common inorganic cations on virus adsorption. The effect of ionic strength was also investigated by varying the salt concentration. Increasing the ionic strength with NaCl from 3 to 4 mM decreased removal (Figure 2). This is likely due to the greater charge screening effect at higher ionic strength, and consequently reduced electrostatic attraction between the negatively charged viruses and the positively charged magnetite nanoparticles. The removal of MS2 by 1 g/L magnetite nanoparticles increased when the divalent calcium ion Ca^{2+} was present. Ca^{2+} was more effective than Na^{+} in promoting virus removal (i.e., 2.7-log removal of MS2 was achieved in the presence of Ca^{2+} compared to less than 0.5-log removal in the presence of Na^{+} alone). This could be attributed to two factors: (1) Ca^{2+} promotes virus coagulation to form complexes, and (2) A small number of negatively charged sites exist on the overall positively charged magnetite surface. Ca^{2+} forms ionic bridges between the few negative charge sites on magnetite surface and those on MS2 capsid.

These results corroborate previous studies reporting that divalent cations enhanced the deposition of MS2 to NOM-coated silica surface and silica (Pham et al., 2009). Apparently, Ca^{2+} promotes electrostatic attraction due to its tendency to form complexes on the adsorbent surface, which bind to negatively charged carboxylate groups on the viral capsid proteins.

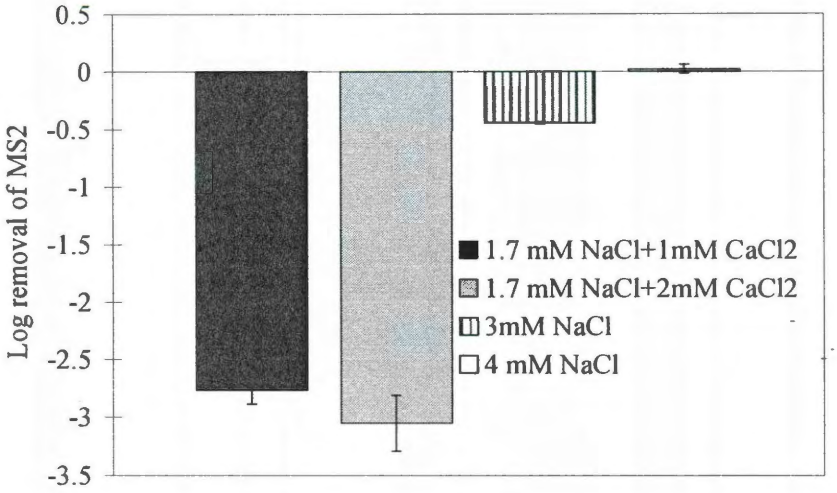

Figure 2. Removal of MS2 by 1 g/L magnetite nanoparticles at pH 6 after 1 h incubation in different electrolyte solutions. Initial virus concentration was 10^6 PFU/mL.

3.2 Membrane Characterization

Polysulfone membranes coated with ~3 mg of nanomagnetite (nFe_3O_4-PSf) had permeability three times lower than the control membrane (PSf) without nFe_3O_4 (Table 1). The decrease in membrane permeability due to the hydraulic resistance imposed by the nanomagnetite coating layer represents a concern on the higher energy requirement for membrane filtration. On the other hand, the nFe_3O_4 membrane was slightly more hydrophilic than the control sample with a contact angle 7% smaller than that of the control PSf membrane. This decrease in hydrophobicity has potential benefits in preventing membrane fouling (Cheryan, 1998).

Table 1. Basic Properties of the PSf and nFe_3O_4-PSf membranes. (Values presented as average ± range; n =2)

	PSf	**nFe_3O_4-PSf**
Permeability (LMH/psi)	222 ± 6.4	70 ± 5.1
Contact Angle (°)	70 ± 6.8	65 ± 6.3

3.3 Virus Adsorption onto Nanomagnetite-Coated (nFe_3O_4-PSf) Membranes

Significant virus removal (> 99.99% with average virus log removal of 4.4 ± 0.5 (n=9)) was observed when 3 mL of viral suspension was filtered through membranes coated with magnetite nanoparticles, a dramatic improvement relative to the control membranes without magnetite, which only showed less than 0.5 log removal (Figure 3). The removal of virus by nano-Fe_3O_4-PSf membrane is a novel significant finding.

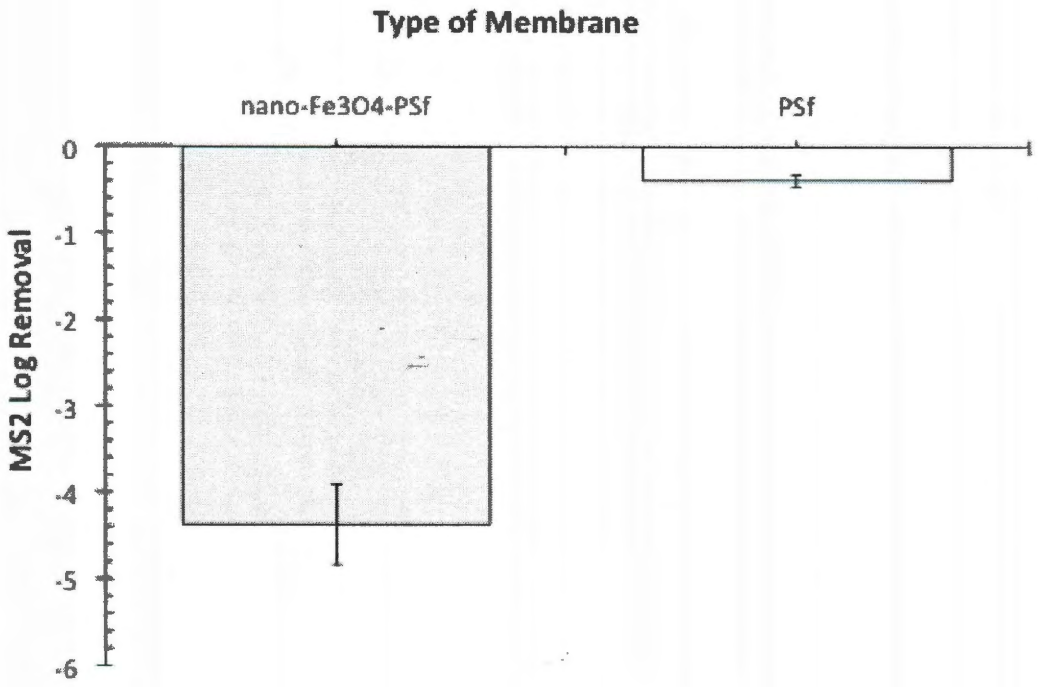

Figure 3. Virus removal by the addition of nano-Fe3O4-PSf Membranes. Initial virus concentration ~107 PFU/mL.

Two mechanisms of virus removal were considered (1) size exclusion through the nanomagnetite coating (potentially clogging pores or decreasing the pore size), and (2) electrostatic adsorption of the viruses to the magnetite nanoparticles. A continuous flow experiment was carried out to evaluate n-Fe_3O_4-PSf membrane performance for MS2 removal and to determine the main virus removal mechanism. A 4.5-log removal was initially observed (Figure 4a). The MS2 breakthrough curve is presented in Figure 4b. The allowable breakthrough concentration (representing 4-log virus removal) was reached after 18 mL of filtrate was collected. The decreasing removal efficiency and increasing effluent virus concentration with increasing

cumulative filtrate volume suggests that adsorption likely via electrostatic interaction instead of size exclusion is the major removal mechanism. Adsorptive removal efficiency decreased as more and more adsorption sites were occupied. Size exclusion, which would result in a stable or increasing (due to pore blockage) removal, did not seem to be the predominant mechanism.

The permeate volume at breakthrough corresponds to approximately 25 min of filtration time at a typical permeate flux of 50 gallons per square foot per day (GFD), suggesting that the n-Fe_3O_4 coating approach could be sustainable if the magnetite nanoparticles can be regenerated at each backwash by adjusting the solution chemistry (e.g., pH) of the backwash water. Adsorption capacity can be defined as the number of infectious virus particles (PFU) adsorbed per gram of magnetite nanoparticles in solution (Gutierrez et al., 2009). Accordingly, the adsorption capacity for MS2 in a 0.1 M bicarbonate buffer solution (pH 8.3) for 4.5-log (average Log removal of MS2 in our continuous flow experiment) was 3.3×10^9 PFU/g.

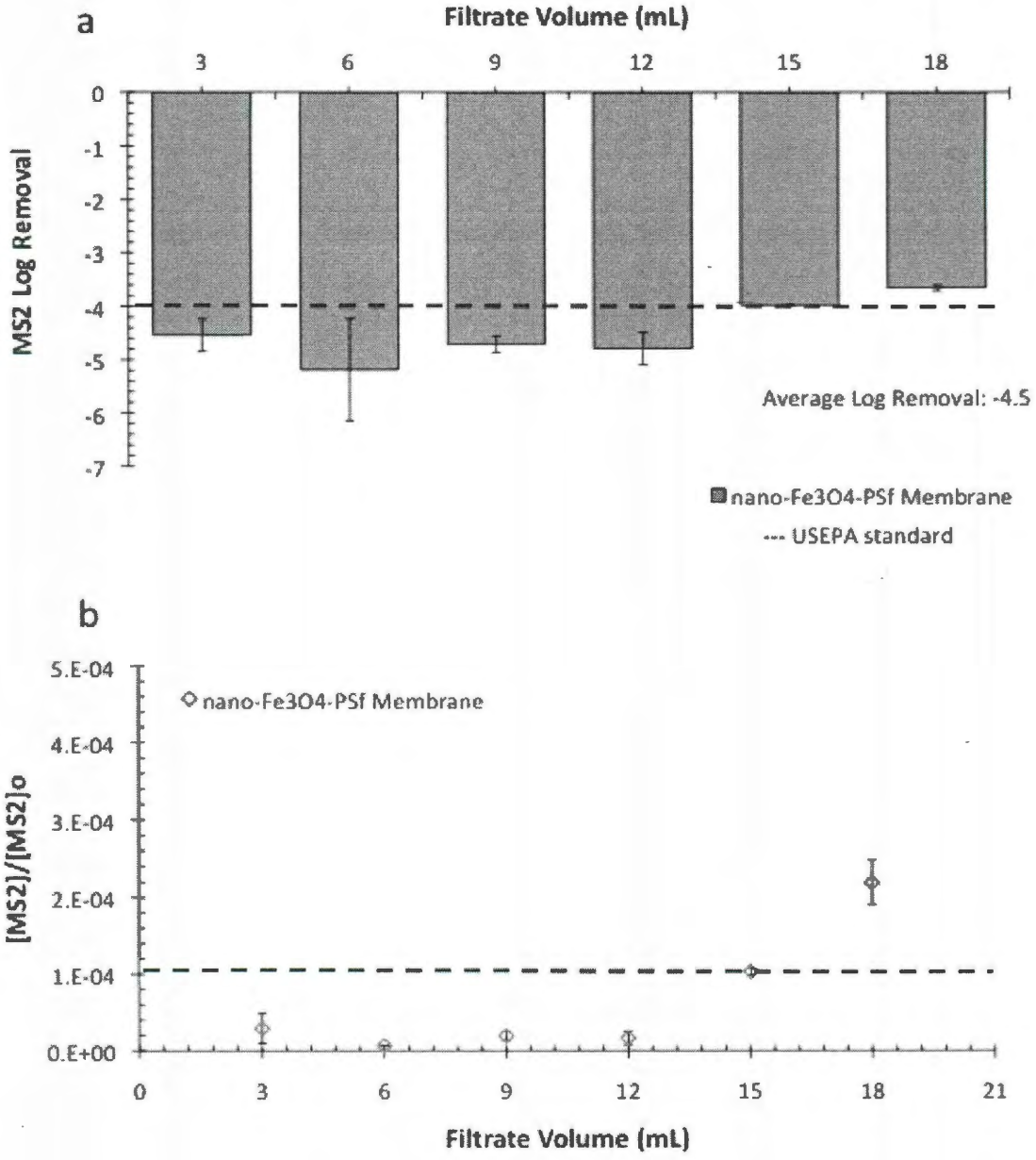

Figure 4. a) Removal of MS2 by nFe_3O_4-PSf Membranes. Initial virus concentration ~10^7 PFU/mL. b) MS2 breakthrough curve from flow-through experiments with nFe_3O_4-PSf Membranes. The 4-log removal USEPA requirement is depicted as a dotted line.

3.4 Analysis of Iron in the Filtrate and Implications for Long-term Performance

The average total Fe in the membrane permeate (after filtration of 3 mL MS2 suspension) was 10.6 ± 0.006 μg/L, corresponding to 0.00106% over the total estimated amount of nanomagnetite coated on the membrane (3 mg). The effluent iron concentration was significantly below the 0.3 mg/L secondary standard for drinking water, which was set to prevent aesthetic impacts on color and taste.

During the operation of a membrane system, the membrane surface and the pore wall is subject to hydraulic shear, which can potentially disturb the nanomagnetite coating layer and release magnetite nanoparticles. Therefore, superficial and transversal rinsing protocols were performed to remove loosely attached magnetite nanoparticles before filtration experiments. These two rinsing protocols simulate the hydraulic condition encountered in cross-flow and dead-end filtration, respectively. As shown in Table 2, MS2 removal after both rinsing protocols decreased compared to that without rinsing, suggesting the loss of some magnetite nanoparticles during the rinse. However, the rinsed membranes were still able to achieve greater than 4-log removal of MS2, indicating that an effective coating layer remained. These results suggest that the simple coating procedure used in this study may be effectively applied to industrial membrane units. Long term durability testing is needed to determine the lifetime of the coating.

Table 2. Virus removal by nFe_3O_4-PSf membranes with different rinsing methods.

Rinsing Membrane Method	Log MS2 Removal
No rinse	6.0
Superficial	4.3
Superficial/Transversal	4.4 ± 0.5

CHAPTER 4:

CONCLUSIONS

In agreement with the literature, we showed that magnetite successfully removes viruses by adsorption. Furthermore, polysulfone membranes coated with magnetite nanoparticles were effective in removing bacteriophage MS2, potentially obviating the need for pre- or post-treatment to remove viruses in a membrane based system. Commercial polysulfone membranes are notable for their widespread application in water filtration. Cartridges of polysulfone membranes coated with nano-Fe_3O_4 particles may be an option to consider as a point- of-use devices and modification to existing membrane filtration processes to remove viruses from water. Advantages of nanomagnetite coating of polysulfone membranes include the simple coating protocol, avoidance of harmful disinfection byproducts, and negligible iron leaching into the filtrate. Due to the limited virus adsorption capacity of membranes prepared in this fashion, regeneration of the nanomagnetite material is necessary for this approach to be sustainable.

Although these results are promising, much research remains to be conducted to determine the feasibility of such membranes to treat different source waters. Key challenges include coating methods that lower the filtration energy requirement, and backwash processes (e.g., with a basic solution) to regenerate adsorption capacity.

REFERENCES

Atherton, J. G., and Bell, S. S. (1983). Adsorption of viruses on magnetic particles--I. Adsorption of MS2 bacteriophage and the effect of cations, clay and polyelectrolyte. *Water Research*, 17(8), 943-948.

Badireddy, A. R., Hotze, E. M., Chellam, S., Alvarez, P., and Wiesner, M. R. (2007). Inactivation of Bacteriophages via photosensitization of fullerol nanoparticles. *Environmental Science & Technology*, 41(18), 6627-6632.

Benabbou, A. K., Derriche, Z., Felix, C., Lejeune, P., and Guillard, C. (2007). Photocatalytic inactivation of *Escherichia coli* effect of concentration of TiO_2 and microorganism, nature, and intensity of UV irradiation. *Applied Catalysis B-Environmental*, 76(3-4), 257-263.

Bitton, G., Pancorbo, O., and Gifford, G. E. (1976). Factors affecting the adsorption of polio virus to magnetite in water and wastewater. *Water Research*, 10(11), 973-980.

Borchardt, M. A., Bradbury, K. R., Gotkowitz, M. B., Cherry, J. A., and Parker, B. L. (2007). Human enteric viruses in groundwater from a confined bedrock aquifer. *Environmental Science & Technology*, 41(18), 6606-6612.

Brame, J., and Alvarez P.J.J (2011). Nanotechnology-enabled water treatment and reuse: emerging opportunities and challenges for developing countries, Trends in Food Science & Technology, doi:10.1016/j.tifs.2011.01.004

Brown, J., and Sobsey, M. D. (2009). Ceramic media amended with metal oxide for the capture of viruses in drinking water. *Environmental Technology*, 30(4), 379-391.

Cheryan, M. (1998).Ultrafiltration and Microfiltration Handbook, 2nd edn, CRC Press, Boca Raton.

Cho, M., Chung, H., and Yoon, J. (2005). Different inactivation behaviors of MS2 phage and *Escherichia coli* in TiO_2 photocatalytic disinfection. *Applied and Environmental Microbiology*, 71(1), 270-275.

Chu, Y., Jin, Y., Baumann, T., and Yates, M. V. (2003). Ground water quality: Effect of soil Properties on Saturated and Unsaturated Virus Transport through Columns. *Journal of Environmental Quality*, 32(6), 2017-2025.

Churchhouse, S. (2000). Membrane bioreactors hit the big time – from lab to full scale application. In: Rautenbach, R., Melin, T., Dohmann, M. (Eds.), Membrantechnik, 3. Aachener Tagung, B12. Klenkes Druck und Verlag GmbH, Aachen, 11–17.

Dowd, S. E., Pillai, S. D., Wang, S., and Corapcioglu, M. Y. (1998). Delineating the Specific Influence of Virus Isoelectric Point and Size on Virus Adsorption and Transport through Sandy Soils. *Appl. Environ. Microbiol.*, 64(2), 405-410.

Ehlers, M. M., Grabow, W. O. K., and Pavlov, D. N. (2005). Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Research*, 39(11), 2253-2258.

Elechiguerra, J. L., Burt, J. L., Morones, J. R., Camacho-Bragado, A., Gao, X., Lara, H. H., and Yacaman, M. J. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology* **3**:6.

Fong, T.-T., and Lipp, E. K. (2005). Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools. *Microbiol. Mol. Biol. Rev.*, 69(2), 357-371.

Furukawa, D. A Global Perspective of Low Pressure Membranes; National Water Research Institute: Fountain Valley, CA, March 2008.

Griffin, D. W., Donaldson, K. A., Paul, J. H., and Rose, J. B. (2003). Pathogenic Human Viruses in Coastal Waters. *Clin. Microbiol. Rev.*, 16(1), 129-143.

Gutierrez, L., Li, X., Wang, J., Nangmenyi, G., Economy, J., Kuhlenschmidt, T. B., Kuhlenschmidt, M. S., and Nguyen, T. H. (2009). Adsorption of rotavirus and bacteriophage MS2 using glass fiber coated with hematite nanoparticles. *Water Research*, 43(20), 5198-5208.

Hagen, K. (1998). Removal of particles, bacteria and parasites with ultrafiltration for drinking water treatment. *Desalination*, 119(1-3), 85–91.

Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y., Yao, Y., Huang, D., and Hao, B. (2008). Toxicological effect of ZnO nanoparticles based on bacteria. *Langmuir*, 24(8), 4140-4144.

Hurst, C. J., Gerba, C. P., and Cech, I. (1980). Effects of environmental variables and soil characteristics on virus survival in soil. *Appl. Environ. Microbiol.*, 40(6), 1067-1079.

ISO10705-1. (1995). "ISO 10705-1 Water quality-detection and enumeration of bacteriophages Part 1: Enumeration of f-specific RNA bacteriophages." International Standard Organization, Geneve, Switzerland.

John, D. E., and Rose, J. B. (2005). Review of Factors Affecting Microbial Survival in Groundwater. *Environmental Science & Technology*, 39(19), 7345-7356.

Kennedy, J. E., Wei, C. I., and Oblinger, J. L. (1986). Methodology for enumeration of coliphages in foods. *Applied and environmental microbiology*, 51(5), 956-962.

Keswick, B. H., Gerba, C. P., DuPont, H. L., and Rose, J. B. (1984). Detection of enteric viruses in treated drinking water. *Appl. Environ. Microbiol.*, 47(6), 1290-1294.

Kikuchi, Y., Sunada, K., Iyoda, T., Hashimoto, K., and Fujishima, A. (1997). Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. *Journal of Photochemistry and Photobiology A: Chemistry*, 106(1-3), 51-56.

Langlet, J., Ogorzaly, L., Schrotter, J.-C., Machinal, C., Gaboriaud, F., Duval, J. F. L., and Gantzer, C. (2009). Efficiency of MS2 phage and Q[beta] phage removal by membrane filtration in water treatment: Applicability of real-time RT-PCR method. *Journal of Membrane Science*, 326(1), 111-116.

Lee, S.-H., and Kim, S.-J. (2002). Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. *Water Research*, 36(1), 248-256.

Li, M., Zhu, L., and Lin, D. (2011). Toxicity of ZnO Nanoparticles to *Escherichia coli*: Mechanism and the Influence of Medium Components. *Environmental Science & Technology*, 45(5), 1977-1983.

Liau, S. Y., Read, D. C., Pugh, W. J., Furr, J. R, and Russell, A. D. (1997). Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. *Lett. Appl. Microbiol.*, 25(4), 279-283.

Liga, M. V., Bryant, E. L., Colvin, V. L., and Li, Q. (2011). Virus inactivation by silver doped titanium dioxide nanoparticles for drinking water treatment. *Water Research*, 45(2), 535-544.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5(5), 607-625.

Moore, R. S., Taylor, D. H., Sturman, L. S., Reddy, M. M., and Fuhs, G. W. (1981). Poliovirus Adsorption by 34 Minerals and Soils. *Appl. Environ. Microbiol.*, 42(6), 963-975.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. P., and Yacaman, M. Y. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology* 16(10), 2346-2353.

Page, K., Palgrave, R. G., Parkin, I. P., Wilson, M., Savin, S. L. P., and Chadwick, A.V. (2007). Titania and silveretitania composite films on glass-potent antimicrobial coatings. *Journal of Materials Chemistry,* 17(1), 95-104.

Pal, S., Tak, Y. K., and Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram–negative bacterium *Escherichia coli. Appl. Environ. Microbiol.* 73(6), 1712-1720.

Pham, M., Mintz, E. A., and Nguyen, T. H. (2009). Deposition kinetics of bacteriophage MS2 to natural organic matter: Role of divalent cations. *Journal of Colloid and Interface Science*, 338(1), 1-9.

Pronk W., Zurbrugg, C., Swartz C. (2009). Descentralized systems for potable water and the potential of membrane technology. *Water Research*, 43(2), 245-265.

Raghupathi, K. R., Koodali, R. T., and Manna, A. C. (2011). Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. *Langmuir*, 27(7), 4020-4028.

Rao, V. C., Waghmare, S. V., and Lakhe, S. B. (1981). Detection of Viruses in Drinking Water by Concentration on Magnetic Iron Oxide. *Applied and environmental microbiology*, 42(3), 421-426.

Ryan, J. N., Harvey, R. W., Metge, D., Elimelech, M., Navigato, T., and Pieper, A. P. (2002). Field and Laboratory Investigations of Inactivation of VirusesField and Laboratory Investigations of Inactivation of Viruses (PRD1 and MS2) Attached to Iron Oxide-Coated Quartz Sand. *Environmental Science & Technology*, 36(11), 2403-2413.

Schijven, J. F., and Hassanizadeh, S. M. (2000). Removal of Viruses by Soil Passage: Overview of Modeling, Processes, and Parameters. *Critical Reviews in Environmental Science and Technology*, 30(1), 49 - 127.

Singh, M., Singh, S., Prasada, S., Gambhir, I. S. (2008). Nanotechnology in medicine and antibacterial effect of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures* 3(3), 115-122.

Sondi, I., Sondi, B. S. (2004). Silver nanoparticles as antimicrobial agent: A case study on *E-coli* as a model for Gram-negative bacteria. *J. Coll. Interf. Sci.*, 275(1), 177-182.

Theron, J., Walker, J. A., Cloete, T. E. (2008). Nanotechnology and Water Treatment: Applications and Emerging Opportunities. *Critical Reviews in Microbiology,* 34(1), 43-69.

Tiwari, D. K., Behari, J., Sen, P. (2008). Application of Nanoparticles in Waste Water Treatment. *World Applied Sciences Journal*, 3(3), 417-433.

VanDuin, J. (1988). The Bacteriophage. *Plenum*, New York.

Vivier, J. C., Ehlers, M. M., and Grabow, W. O. K. (2004). Detection of enteroviruses in treated drinking water. *Water Research*, 38(11), 2699-2705.

Wang, J., Guo, J., Santiwong, S. R., Waite, T. D. (2007). Characterization of floc size and structure under different monomer and polymer coagulants on microfiltration membrane fouling. *Journal of Membrane Science*, 321(2), 132–138.

Watts, R. J., Kong, S. H., Orr, M. P., Miller, G. C., Henry, B. E. (1995). Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. *Water Research*, 29(1), 95-100.

Wei, C., Lin, W. Y., Zainal, Z., Williams, N. E., Zhu, K., Kruzic, A. P., Smith, R. L., Rajeshwar, K. (1994). Bactericidal activity of TiO_2 photocatalysis in aqueous-media towards a solar assisted water disinfection system. *Environmental Science & Technology*, 28(5), 934-938.

World Health Organization (2007). Combating waterborne disease at the household level / International Network to Promote Household Water Treatment and Safe Storage.

Xiao, Y. T., Xu, S. S., Li, Z. H., et al. (2010). Progress of applied research on TiO_2 photocatalysis-membrane separation coupling technology in water and wastewater treatments. *Chinese Sci. Bull.*, 55(14), 1345-1353.

You, Y., Han, J., Chiu, P. C., and Jin, Y. (2005). Removal and Inactivation of Waterborne Viruses Using Zerovalent Iron. *Environmental Science & Technology,* 39(23) 9263-9269.

Yuan, B., Pham, M., and Nguyen, T. H. (2008). Deposition Kinetics of Bacteriophage MS2 on a Silica Surface Coated with Natural Organic Matter in a Radial Stagnation Point Flow Cell. *Environmental Science & Technology*, 42(20), 7628-7633.

Zerda, K. S., Gerba, C. P., Hou, K. C., and Goyal, S. M. (1985). Adsorption of viruses to charge-modified silica. *Appl. Environ. Microbiol.*, 49(1), 91-95.

Zhu, B., Clifford, D. A., and Chellam, S. (2005). Virus removal by iron coagulation-microfiltration. *Water Research*, 39(20), 5153-61.

Zodrow, K., Brunet, L., Mahendra, S., Li, D., Zhang, A., Li, Q., and Alvarez, P. J. J. (2009). Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. *Water* Research, 43(3), 715-723.